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NEPHROLOGY FORUM

Disorders of the epithelial sodium channel: Insights into the regulation of extracellular volume and blood pressure

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CASE PRESENTATIONS

Patient 1. A five-day-old Hispanic boy was the result of an uncomplicated pregnancy. The vaginal delivery and Apgar scores were normal. Neonatal jaundice secondary to ABO incompatibility was treated with phototherapy. The baby had no family history of congenital disorders.

Physical examination disclosed a weight of 2870 g and was unremarkable except for jaundice. Neurologic development was appropriate for age, and there was no sexual ambiguity. Laboratory examination revealed a serum sodium of 128 mEq/liter; potassium, 7.7 mEq/liter; chloride, 87 mEq/liter; and bicarbonate, 18 mEq/liter. The BUN was 24 mg/dl and the serum creatinine 0.4 mg/dl. The white blood cell count was 23,900/mm^3; the hematocrit, 56%; and the total bilirubin, 20.6 mg/dl. Direct bilirubin was 1.3 mg/dl. Blood gas evaluation showed pH, 7.51; PCO_2, 29 mm Hg; and PO_2, 48 mm Hg. He was transferred to the University of Iowa Hospitals.

The baby was treated with 10% glucose and normal saline, insulin infusion, oral sodium polystyrene sulfonate, and 9 alpha fluorohydrocortisone, 0.1 mg orally daily. Subsequent laboratory examination showed a plasma aldosterone of 1218 ng/dl (normal, 5 to 90 ng/dl), and a plasma renin activity of 350 ng/ml/hr (normal, 0.5 to 3.0 ng/ml/hr). The sweat chloride concentration was abnormally high at 152 mM. A genetic screening for 70 common alleles of cystic fibrosis was negative.

The infant's electrolytes stabilized with sodium chloride supplementation, and he proceeded to gain weight normally. Additional testing revealed that the nasal transepithelial voltage was approximately zero mV and showed no amiloride sensitivity. (Normal baseline values for patients this age are more negative than −20 mV.) The response to beta-adrenergic agonists was normal. The patient did not respond to exogenous mineralocorticoid therapy. The urinary sodium excretion was 41 mEq/liter and a simultaneous urinary potassium level was 1 mEq/liter. Oxygen saturation throughout the entire hospital stay was within normal limits. The hyperbilirubinemia resolved with phototherapy. Evaluation of the renin-angiotensin axis in the parents showed completely normal values in the father and marginally elevated plasma aldosterone values in the mother.

Patient 2. A 16-year-old female was referred for hypertension, hypokalemia, and metabolic alkalosis. Two siblings had similar findings. The patient's blood pressure averaged 180/120 mm Hg. The serum potassium was 2.6 mEq/liter; bicarbonate, 30 mEq/liter; and arterial blood pH, 7.45. Despite the hypokalemia, she excreted 80 mEq of potassium per day. Evaluation showed a barely detectable aldosterone secretion rate but normal excretion of other adrenal corticosteroids. Upon dietary salt restriction, she was able to eliminate sodium from her urine normally.

Twenty-nine years later, she developed end-stage renal disease and was begun on dialysis. Shortly thereafter she underwent a cadaveric kidney transplant and currently has excellent renal function with only mild hypertension (140/90 mm Hg). She currently has normal serum potassium and acid-base values.

> *"Nature is nowhere accustomed more openly to display her secret mysteries than in cases where she shows traces of her workings apart from the beaten path."*
>
> WILLIAM HARVEY, 1657

DISCUSSION

DR. JOHN B. STOKES (*Professor of Medicine, Director, Division of Nephrology; Department of Internal Medicine; University of Iowa; and Department of Veterans Affairs Medical Center, Iowa City, Iowa, USA*): The disorders collectively called pseudohypoaldosteronism (PHA) result from an inability of aldosterone to produce its major physiologic effects. The most prominent effect, well-

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Table 1. Characteristics of the three types of pseudohypoaldosteronism

	GFR	BP	Salt wasting	$[HCO_3^-]$	$[K^+]$	Renin	Aldosterone
Type I	↓,N	↓	Yes	↓	↑	↑	↑
Type II	N	↑	No	↓,N	↑	⇊	↓,N
Type III	⇊	↓	Yes	↓	↑	↑	↑

known to all nephrologists, is on the cortical collecting duct (CCD). As illustrated in Figure 1, aldosterone binds to the mineralocorticoid receptor located in the cell cytoplasm, which then becomes activated and translocates to the nucleus. In the nucleus a variety of poorly understood transcription processes become up- or downregulated; these changes subsequently increase the permeability of the apical membrane to sodium by increasing the activity of the epithelial sodium channel (ENaC). The increase in sodium permeability raises the rate of sodium entry across the apical (luminal) membrane. Concomitantly there is an increase in the rate of extrusion of sodium across the basolateral (anti-luminal) membrane by the Na^+/K^+ pump. The resulting increase in potassium entry, a direct consequence of the increase in sodium extrusion, raises the rate of potassium extrusion across the apical potassium channel. Most cells have a potassium ion channel located on the basolateral membrane; this channel allows recycling of potassium and produces no net transfer across the epithelium. The CCD, by having a relatively low basolateral potassium conductance and a very high apical membrane potassium conductance, is ideal for secreting large amounts of potassium into the urine when sodium absorption is substantial. Aldosterone also increases the activity of the apical potassium channel as well as the basolateral membrane Na^+/K^+ pump [1].

Pseudohypoaldosteronism represents a group of disorders that are commonly classified into three types (Table 1). All three are characterized by hyperkalemia and metabolic acidosis. These disorders thus are considered in the general category of type-IV renal tubular acidosis. I plan to focus my discussion on type-I PHA but first will discuss briefly types II and III. Type-III PHA results from a group of acquired disorders that injure the collecting duct, such as medullary cystic diseases or obstructive uropathy. An important hallmark of type-III PHA is a reduced filtration rate.

Type-II PHA is a controversial designation. As Table 1 shows, the patients manifesting this disorder do not invariably have elevated plasma aldosterone and plasma renin levels as one would expect in patients with a "true" pseudohypoaldosteronism. Perhaps the best described phenotype is Gordon's syndrome [2]. The molecular defect is not known. Hypertension and a tendency to low plasma renin and aldosterone levels characterize this group of patients. Thus, there is reason to question whether this group of disorders should be included in the designation pseudohypaldosteronism.

Type-I PHA includes a group of patients represented by today's first case. They are characterized by normal renal function, a tendency to waste salt, hypotension, hyperkalemia, metabolic acidosis, and high circulating renin and aldosterone levels. As exemplified by the index case, these patients tend to be identified very early in life and have life-threatening hyperkalemia as their chief therapeutic challenge. We now understand the major molecular problems that lead to this syndrome.

There are at least two genetic types of type-I PHA: autosomal-dominant and autosomal-recessive. The autosomal-dominant form (or perhaps sporadic, as in today's first case) tends to have a relatively mild phenotype that can be readily controlled by high dietary salt intake and careful attention to potassium balance. Children with this form of PHA tend to outgrow the syndrome and require little or no management after emerging from the first few years of life.

A large fraction of patients with autosomal-dominant and sporadic type-1 PHA appear to have abnormalities in the mineralocorticoid receptor gene. Geller et al showed genetic abnormalities in five kindreds that would be predicted to produce major abnormalities in the mineralocorticoid receptor protein [3]. These abnormalities include frame-shift mutations, abnormal splicing, and premature stop codons. These discoveries point to the critically important role that the mineralocorticoid receptor plays in sodium, potassium, and acid-base balance in early life. These cases also underscore the fact that the epithelial sodium channel can be dysfunctional as a result of abnormalities in gene products responsible for its regulation in addition to abnormalities intrinsic to the gene itself. I will return to this concept later.

The discovery that defects in only one copy of the mineralocorticoid receptor can produce severe imbalance in potassium metabolism in infants came as somewhat of a surprise. The reason why a single abnormal copy (expressed with one normal copy) of the mineralocorticoid receptor produces disease is not clear. The situation contrasts starkly with the recently published results from genetically altered mice that lack the mineralocorticoid receptor [4]. In these studies, heterozygous mice had a normal phenotype, at least with respect to serum potassium levels. The heterozygous pups achieved a normal serum potassium at the expense of a moderately elevated plasma renin activity and serum aldosterone concentration. Thus the reason that human beings with similar genetic defects appear to have a more difficult neonatal course remains an enigma.

The other form of type-I PHA is the autosomal-recessive variety. Chang et al described five kindreds, all involving consanguinity, with major genetic differences in sequences for one of the genes encoding the epithelial

sodium channel (ENaC) [5]. These kindreds, whose affected members all have homozygous defects, exhibit severe phenotypes of salt-wasting, hyperkalemia, and metabolic acidosis.

Molecular nature of ENaC

The discovery and cloning of ENaC [6–8] opened a new chapter in our understanding of electrolyte homeostasis and blood pressure control. For years it has been known that one of the major targets for aldosterone's action was the apical membrane sodium channel of the renal collecting duct. The technique of expressing mRNA in the *Xenopus* oocyte has permitted the cloning of several membrane transport proteins, including the Na^+/Cl^+ cotransporter, the $Na^+/K^+/2Cl^-$ cotransporter, the Na^+/glucose cotransporter, potassium ion channels, and many others. The identification of ENaC, however, was complicated by the fact that it was comprised of three separate gene products, all of which were required for its full expression. Fortunately, expression of a single subunit, the α subunit, provided enough channel activity to permit its cloning and molecular identification [6]. Shortly thereafter, the β and γ subunits of ENaC were identified [7].

The molecular organization of a representative subunit, depicted in Figure 2A, is similar for all three subunits [9–11]. Each subunit of ENaC has two membrane-spanning regions with the amino terminus and the carboxy terminus inside the cell. In addition, a large extracellular loop between the membrane-spanning domains contains glycosylation sites and cysteine-rich regions. The extracellular regions of these subunits are quite large—much larger than is the case for other ion channels. The significance of such a large extracellular domain is not clear, but there is speculation that specific regions can interact with substances in the extracellular environment and modify the function of ENaC. A region of the extracellular loop near the second membrane-spanning domain appears to act as a selectivity filter and might contain a binding site for amiloride [12].

The three subunits come together to form a channel in ways that are not completely understood. Figure 2B is an oversimplified schematic diagram of how each of the three subunits might interact to form a channel. It is reasonably clear from the available data that the membrane-spanning domains contribute in some fashion to the pore-forming region of the molecule. The stoichiometry of the complex is almost certainly not $1\alpha:1\beta:1\gamma$ subunit. The actual stoichiometry is unclear, but two views prevail. One view would have the stoichiometry fixed at four subunits ($2\alpha:1\beta:1\gamma$) or perhaps a multiple thereof [13, 14]. Another view would have the stoichiometry fixed at $3\alpha:3\beta:3\gamma$ subunits [15, 16]. Whether the subunits always assemble with the same stoichiometry is also open to question. Good evidence indicates that they can assemble with different stoichiometries to perform substantially different functions [17]. The fact that the relative amount of messenger RNA for each of the subunits can vary widely in various sites, such as lung, kidney, colon, urinary bladder, and mechanoreceptors [18–21], raises the possibility that different stoichiometries can impart different functional consequences.

The ENaC/DEG superfamily

These ENaC subunits are part of a large family of molecules that can form ion channels and that are expressed in a wide variety of organisms from the primitive nematode *C. elegans* to *Homo sapiens.* Figure 3 shows a portion of the known "family tree." With the exception of the ENaC molecules, the function of these proteins is poorly understood. Those in *C. elegans* might be mechanosensors, because mutations in these proteins cause the nematodes to fail to respond to touch and to develop degenerative changes in the neurons involved in touch [22]. Family members from *Drosophila* might have a similar function [23]. The function of the acid-sensing branch of the family remains unclear, although some researchers have speculated that this branch is involved in taste and integrated neural functions [22, 24]. Perhaps individual members of the ENaC family are used for mechanosensation and taste in mammals [20, 21, 25] in addition to their role in forming the classic epithelial sodium channel.

Researchers generally agree that each member of this family can function as an ion channel. This conclusion derives from four lines of reasoning. First, the topology for each of the family members appears to be similar, and classic ENaC is certainly an ion channel. Second, mutations in analogous channels in *C. elegans* produce cell swelling, as if a sodium channel were inappropriately activated [26]. Third, certain point mutations introduced into apparently non-conducting family members can induce amiloride-sensitive ion channel activity [27]. Finally, the most distant family member, the FMRFamide-activated channel from the snail (FaNaCh), can be activated by an external peptide ligand [28], and the entire group of acid-sensing channels can be activated by varying degrees of extracellular acidification. Taken together, these facts suggest that this family of proteins can act as ion channels under the appropriate circumstances. In at least some cases, this ion channel capability can transmit physical information (pH, stretch) from the outside of the cell to the inside.

Mutations in ENaC causing PHA

All the mutations in ENaC that have been identified in patients with PHA are recessive (Fig. 4, Table 2). Most of the mutations produce severe physical disruption of the affected subunit. The known truncations and frameshifts cause loss of large sections of the molecule, including one or both membrane-spanning domains. It

is not surprising that such major perturbations produce a severely dysfunctional channel in the homozygous state. In the heterozygous state, these disruptions do not cause detectable clinical disease. Thus, in contrast to the defect in the mineralocorticoid receptor, in which heterozygosity can produce disease in the neonate, having one normal copy of the three ENaC subunits is sufficient to maintain nearly normal potassium homeostasis. It is important to note that disruptions in any one of the three subunits produces the PHA phenotype. This clinical observation underscores the importance of each of the three subunits to the overall functioning of the channel.

Not all the mutations known to produce PHA are frameshifts or truncations. Two of them produce a single amino acid change. Such mutations often can lend considerable insight into regions of the molecule that are important for its function. A mutation in the β subunit where the glycine at position 37 is mutated to a serine (G37S) provides some potentially important lessons. This region is located on the intracellular side of the first membrane-spanning domain. This glycine is conserved in each member of the family except the acid-sensing clan and the FaNaCh loner. Reproducing this mutation in the oocyte expression system creates an ion channel in which the ability to transport sodium is significantly impaired [29]. It is interesting that when imposed on the β or the γ subunit, the mutation produces an approximate 50% reduction in the ability to transport sodium in the oocyte expression system. In contrast, when placed in the α subunit, this mutation reduces the capacity for sodium transport by about 80%. The reason that this mutation produces a more severe dysfunction of the α subunit is not clear.

The mechanism whereby the G37S mutation produces a dysfunctional channel has been investigated by Gründer et al [29]. One can postulate two basic mechanisms for defective channel function: (*1*) an inability of the cellular machinery to deliver the channel protein to the cell surface, that is, defective protein trafficking, and (*2*) gating of the channel, that is, the ability of the channel to open and/or close properly. It appears that the G37S mutation does not impair the protein's ability to reach the surface of the *Xenopus* oocyte. Rather, it is likely that this mutation somehow impairs the normal ability of ENaC to open. The reason for the defective gating is not yet known.

A second instructive missense mutation causing PHA was recently reported. All ENaC family members have two regions in the extracellular domain that are rich in cysteine residues. Sixteen of these cysteine residues are conserved among the family members, so the possibility exists that these residues are critical for the structure or function of these proteins. Firsov and coworkers undertook a systematic mutational study of these residues and discovered that four residues, when mutated to serine, produced striking reductions in the magnitude of the sodium transport [30]. (Interestingly, six mutations produced a significant increase in the magnitude of the sodium transport.) In each of the mutations that produced a decrease in the sodium current, a similar reduction occurred in the amount of sodium channel protein present on the surface of the oocyte. These studies suggest that the cysteine residues in the extracellular domains are necessary for delivering an intact sodium channel to the surface of the cell. The precise mechanisms involved in this process are not clear. The currently available experimental data offer little evidence for intersubunit disulfide bonding. Rather, intrasubunit disulfide bonding probably imparts the specific conformations necessary for adequate delivery to the cell surface [30].

Experimental models of PHA

After the α-ENaC gene was cloned, a logical experiment was to determine the consequences of eliminating this gene product in an intact animal. Most investigators in the field expected that animals lacking this subunit would have hyperkalemia and a salt-losing tendency as the prominent phenotype. It was not clear whether other important phenotypes existed nor whether this gene played a role in normal development.

The results of disrupting the α-ENaC gene in mice (that is, the knockout mouse) were surprising. The mice died within 36 hours of being born [31]. However, they died of respiratory distress and pulmonary edema because they were unable to clear fluid from the lungs after birth. It is important to note that organogenesis was normal and no fetal wastage was seen; the percent of −/− mice born was similar to that predicted (25%). Thus, the α-ENaC gene product was not necessary for normal organ development but was critical for making a transition from fetal to neonatal life. The importance of α-ENaC in pulmonary function was beginning to be realized by a number of investigators, as several groups had noted substantial amounts of α-ENaC mRNA in the lung. It is now clear that the expression of this gene product in the lung is greatly increased in the two to three days immediately prior to birth [19, 32, 33].

These experiments demonstrate important differences between the function of α-ENaC in mice and humans. Human beings with a completely disrupted α-ENaC gene product have survived into adulthood, albeit with hyperkalemia and salt wasting [5]. The explanation for this difference is not entirely clear, but it seems most likely that humans have a redundancy that allows for partial replacement of the α-ENaC function in the lung. This redundancy need not be robust. Even a small amount of α-ENaC is capable of rescuing mice that completely lack this protein [34].

Mice with a completely disrupted β- and γ-ENaC gene product have now been constructed [35, 36]. They share

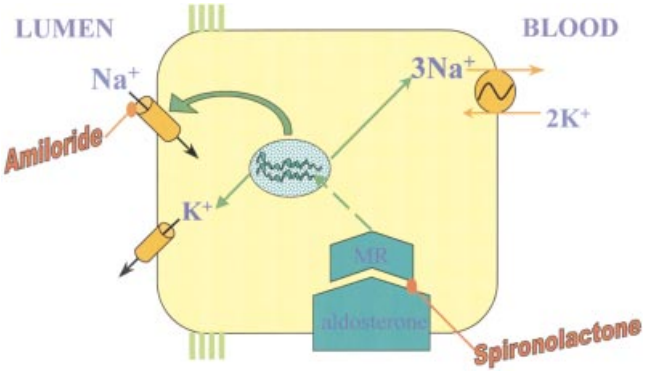

Fig. 1. Schematic of the action of aldosterone on the principal cell of the cortical collecting duct. The major action is to increase the permeability of the Na^+ channel on the apical membrane; this channel is inhibited by amiloride and triamterene. MR, mineralcorticoid receptor.

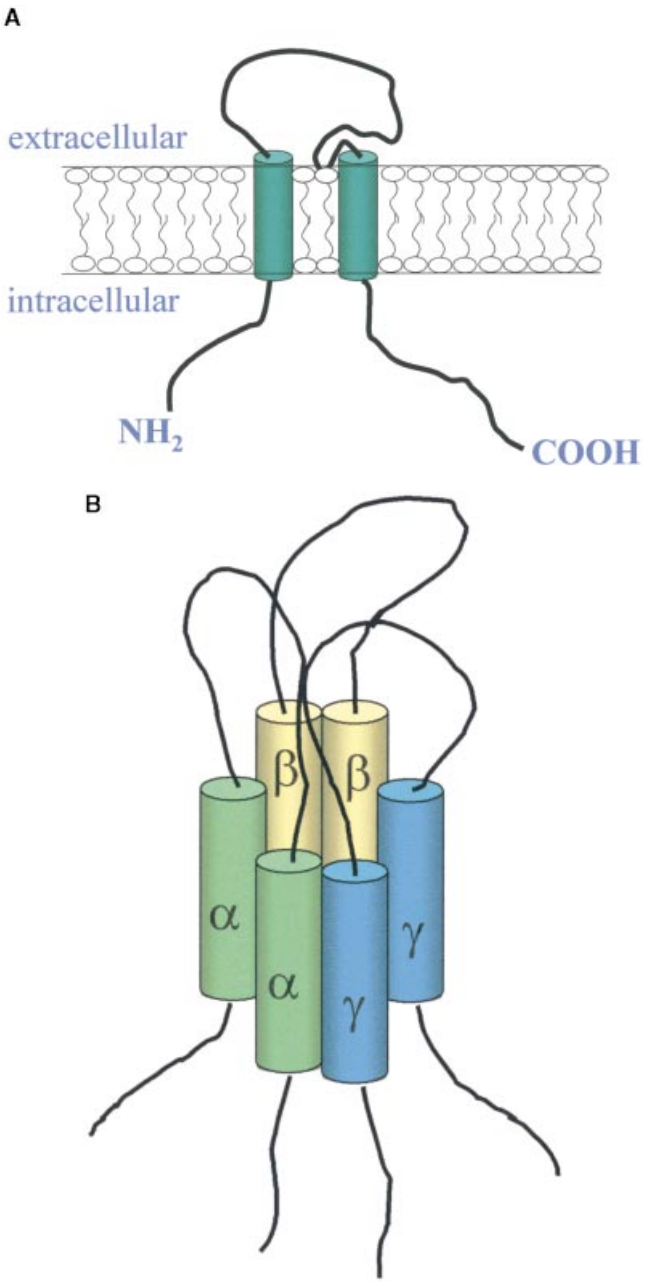

Fig. 2. (*A*) Diagram of a single subunit of ENaC. The general features, two membrane-spanning domains, a large extracellular loop, and intracellular amino- and carboxy-termini, are typical of all members of this family. (*B*) Schematic drawing indicating arrangement of α-, β-, and γ-ENaC into one Na^+ channel. The actual channel complex consists of more than the number of subunits shown here.

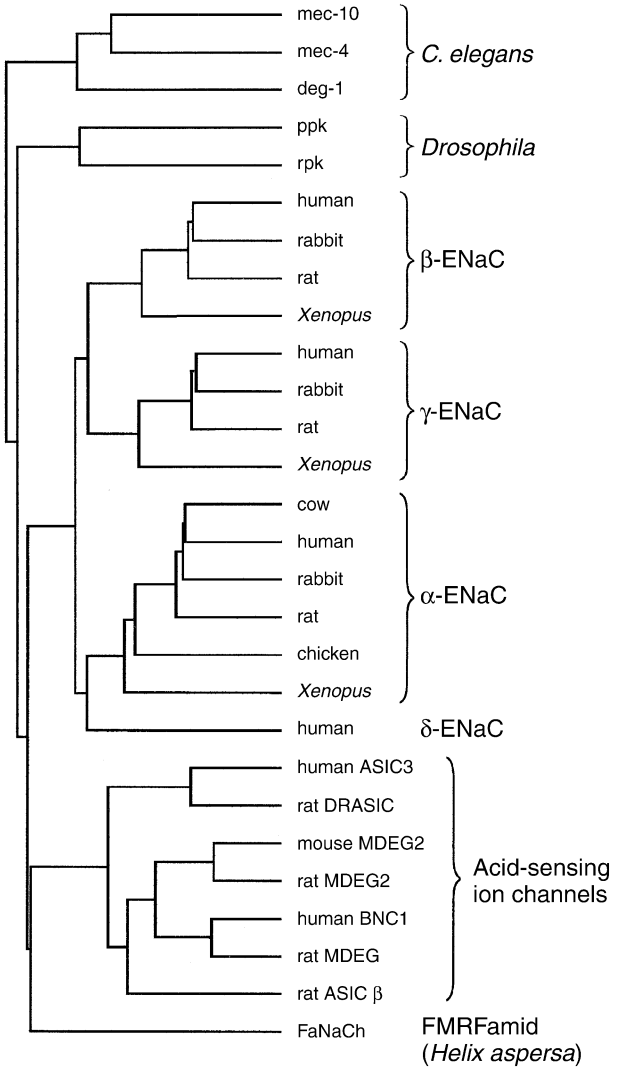

Fig. 3. The members of the ENaC/DEG family.

certain features with the α-ENaC knockout mice (Table 3). They have normal organ development, no fetal wastage, and all mice die within the first two to three days of life. The difference between the β- and γ-ENaC knockout mice and the α-ENaC knockout mice is in the mode of death. The β- and γ-ENaC knockout mice die of severe hyperkalemia and do not exhibit signs of respiratory distress within the first two days of life [35, 36]. Thus, the ENaC gene complex is not required for organ development but is essential for the transition to extrauterine life.

Why is there a phenotypic difference (in mice) between disruption of the α-ENaC gene and the β- and

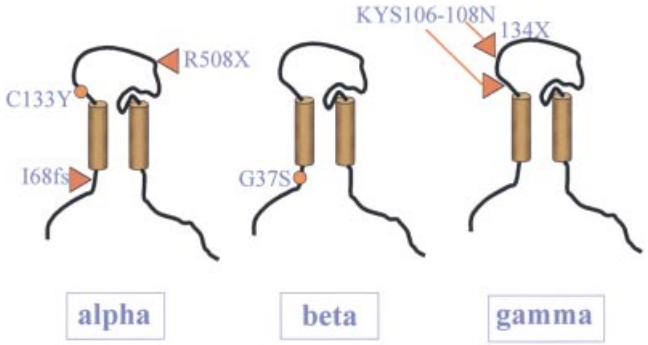

Fig. 4. Mutations in ENaC subunits causing pseudohypoaldosteronism. Triangles indicate premature stop codons or nonsense mutations. Dots indicate missense mutations causing one amino acid substitution. Further details on these mutations are shown in Table 2.

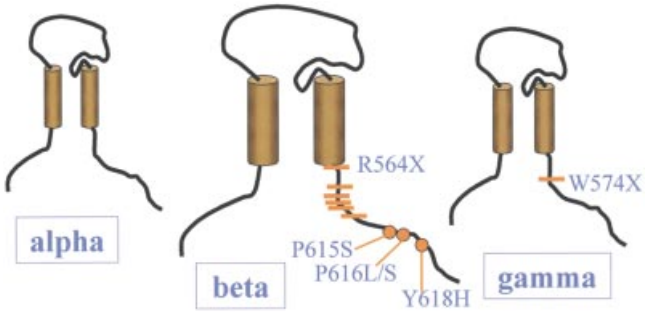

Fig. 5. Mutations in ENaC subunits causing Liddle's syndrome. Short lines represent premature stop codons or nonsense mutations. Dots indicate missense mutations causing one amino acid substitution. Further details on these mutations are shown in Table 4.

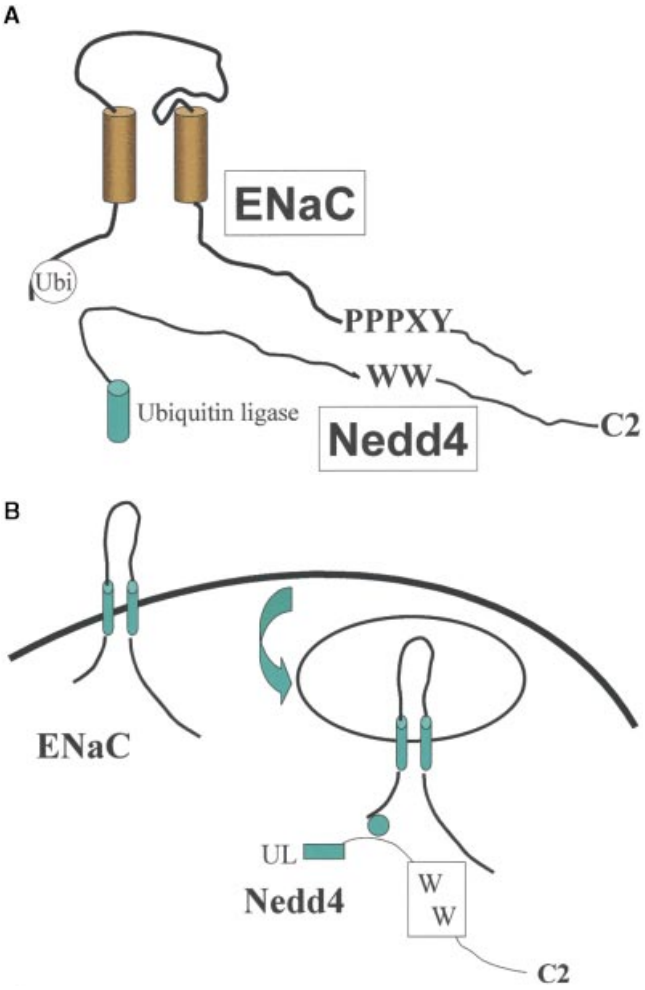

Fig. 6. (*A*) Interaction of one ENaC subunit with Nedd4, a protein involved in regulating surface expression. (*B*) Endoctyosis of a single ENaC subunit as a result of its interaction with Nedd4 and its ubiquitination (represented by a circle).

γ-ENaC genes? The answer to this question is not clear. One possible explanation why the β- and the γ-ENaC knockout mice do not have respiratory distress might be the way the channel complex is formed in the lung. We know that there is relatively more α-ENaC mRNA in the lung than in other organs [19, 37]. We also know that α-ENaC, when expressed alone in oocytes, can form a sodium channel, albeit with a much lower activity than when all three subunits are expressed together [7, 38]. The α/γ-ENaC and α/β-ENaC channels are more active than are the α-ENaC channels, but β/γ-ENaC channels are non-functional. A unifying hypothesis regarding the phenotypic difference between these knockout mice could be constructed as follows. A normal level of α-ENaC expression in the lung can combine with β- or γ-ENaC to form channel activity sufficient to reabsorb lung fluid after birth. However, these α/γ-ENaC or α/β-ENaC channels (in the kidney) are insufficient to provide adequate potassium homeostasis.

This hypothesis might not be complete. Recent work from our laboratory has demonstrated that the developmental expression of the three ENaC subunits is not the same in each organ. Whereas the expression of α-ENaC in the lung increases greatly in the two to three days before birth (relative to β- and γ-ENaC), its expression in the kidney and colon is more gradual and quantitatively less [18, 19]. Thus, a relatively large amount of α-ENaC in the lung might be able to prevent a pulmonary phenotype in the β- and γ-ENaC knockout mice. However, the more modest amounts of α-ENaC in kidney might not be sufficient to prevent the lethal hyperkalemia.

Why can humans survive disruption of any one of the three ENaC genes and mice not? The pundits will quickly point out that mice are different from human beings. However, a more considered view should include the possibility that humans might have a redundant molecule—as yet undiscovered or unrecognized—that can function as a partial surrogate for the defective ENaC subunit. The amount of the putative surrogate protein, or the degree to which it can fully substitute, need not be great. A recently published set of experiments demon-

Table 2. Mutations in ENaC subunits causing pseudohypoaldosteronism

Subunit	Mutation	In vitro expression	Inheritance	Comments	Reference
α-ENaC	I68fs		Recessive		[5]
α-ENaC	R508X		Recessive		[5]
α-ENaC	C133Y	<10% control	Recessive	Temperature sensitive	[30]
β-ENaC	G37S	40% control	Recessive		[5]
γ-ENaC	KYS106–108N; 134X		Recessive		[86]

Abbreviations are: I, isoleucine; R, arginine; C, cysteine; G, glycine; K, lysine; Y, tyrosine; S, serine; N, asparagine; X, stop codon; fs, frameshift mutation.

strated that mice engineered to have very low levels of β-ENaC survive with normal pulmonary function and electrolytes [39]. The PHA phenotype was detectable only after dietary salt restriction.

The general lessons that we can take away from the combination of human and mouse genetic disruption of ENaC are that all three ENaC genes are necessary for normal existence, and that humans and mice do not have to have a fully expressed complement of subunits to sustain a relatively normal existence. As is the case for many other gene products, a small fraction of the normal complement often can ensure a relatively normal existence. However, such defects could predispose to hyperkalemia and metabolic acidosis, particularly under circumstances of dietary salt restriction.

Liddle's syndrome

Today's second patient was originally reported by Grant Liddle in 1963 [40] and revisited in 1994 [41]. She displays the features of the syndrome subsequently named after the investigator who originally described it. Liddle's original description is a remarkably complete and lucid evaluation of a metabolic problem that defied molecular explanation for more than 30 years. One of the key features of this disorder is that the mineralocorticoid antagonist spironolactone had no effect on the sodium balance, potassium secretion rate, or on the blood pressure. However, the diuretic triamterene caused an increase in urinary sodium excretion and a reduction of urinary potassium excretion with an increase in serum potassium concentration. To this day, the diuretics triamterene and amiloride form the cornerstone of the treatment of patients with Liddle's syndrome.

The cloning of the ENaC subunits was the final step that permitted the discovery that Liddle's syndrome is caused by genetic defects in specific regions of the β- or γ-ENaC. This discovery was facilitated by extensive prior work on the detailed mechanism of potassium secretion by the collecting duct, a clear understanding of the action of the diuretics amiloride and triamterene, and the understanding of the action of aldosterone [42]. This critical mass of information, schematized in Figure 1, permitted the development of specific hypotheses to test the idea that defects in ENaC or in its regulation caused the Liddle's phenotype.

Pathophysiology of Liddle's syndrome

Shimkets et al discovered that the genetic defect in the original Liddle's syndrome kindred was a mutation in β-ENaC [43]. This discovery has stimulated an intense interest in understanding the precise molecular events linking this mutation to enhanced sodium absorption by the CCD. The original kindred has a mutation that produces a truncation in β-ENaC such that the entire intracellular carboxy terminus is not synthesized. It is important for us to remember that affected family members have one normal gene copy and one abnormal gene copy, that is, they are heterozygous and the disorder is autosomal-dominant. This mutation fits the classic description of a "gain-of-function mutation." The loss of the C-terminus therefore must result in the loss of a regulatory function that decreases the permeability of the ENaC complex. This loss of a negative regulatory function thus produces a molecule with greater function (that is, higher sodium permeability). Figure 5 and Table 4 demonstrate the known mutations in ENaC that produce Liddle's syndrome. At least six kindreds have truncations of the C-terminus of β-ENaC, and another kindred has a similar truncation of the C-terminus of γ-ENaC. To date, no patients have been reported with Liddle's syndrome caused by similar truncations of α-ENaC.

An internally consistent correlation exists between the phenotype of patients with Liddle's syndrome and the function of mutations of ENaC when expressed in oocytes. For example, consistent with the expectation that the Liddle's mutation should increase sodium transport, truncation of the C-terminus of either β- or γ-ENaC produces higher sodium currents in oocytes than does the full-length protein [44, 45]. The truncation of β-ENaC also produces an increase in sodium current when expressed in MDCK cells [45]. These results provide a solid rationale for using the oocyte expression system to unravel at least some of the ENaC regulatory systems. As Table 4 shows, wherever Liddle's mutations have been expressed in oocytes, they have produced an increase in sodium transport. In sum, all data are consistent

Table 3. Effects of complete disruption of one of the three ENaC subunits in mice

Disrupted subunit	Intrauterine development	Survival of −/− mice	Cause of death	Reference
α-ENaC	Normal	<36 hours	Pulmonary edema	[31]
β-ENaC	Normal	<48 hours	Hyperkalemia	[36]
γ-ENaC	Normal	<36 hours	Hyperkalemia	[35]

Table 4. Mutations of ENaC causing Liddle's syndrome

Subunit	Mutation	In vitro expression	Inheritance	References
Frameshift and premature stop codons				
β	R564X	>3x control	Dominant	[43, 44, 73]
β	AV579del→582X	3.5x control	Dominant	[87]
β	Q589X	ND		[43]
β	T592fs→605X	ND		[43, 72]
β	R594fs	ND		[43]
β	P595fs→604X	ND	Dominant	[88]
β	R597fs→607X	ND	Dominant	[89]
γ	W574X	7x control	Dominant	[90]
Missense mutations				
β	P615S	ND	Dominant	[91]
β	P616L	9x control	Dominant	[47]
β	P616S	ND	Dominant	[49]
β	P616L	ND	Dominant	[49]
β	Y618H	2.5x control	Dominant	[48]

Abbreviations are: R, arginine; A, alanine; V, valine; Q, glutamine; T, threonine; P, proline; W, tryptophan; L, lysine; S, serine; H, histidine; Y, tyrosine; X, stop codon; del, deletion; fs, frameshift. Numbering of the amino acids is based on the rat sequence. The human sequence of the β subunit contains two extra residues upstream of the region where the point mutations exist. Thus, the actual position of these mutations in the human sequence requires adding 2 to the reported number (accession numbers L36593, X87159, X77932).

with the conclusion that these mutations are, in fact, the primary cause of Liddle's syndrome. Figure 5 also demonstrates that no known mutations in α-ENaC cause Liddle's syndrome. There may be a reason for this: truncation of the C-terminus of the α subunit does not increase the sodium current in oocytes [44, 45].

Liddle's mutations could increase the sodium transport rate by two general mechanisms. First, a region of the C-terminus could be involved in the gating properties of the channel. This hypothesis presumes that a portion of this region contains information that affects the physical properties of the channel such as its conductance or its kinetics. The second possibility is that a region of this C-terminus regulates the number of channels residing in the apical membrane. It is highly likely that ENaC is inserted into and retrieved from the apical membrane, but the cellular machinery responsible for this activity is not understood.

A few years ago, two groups significantly advanced our understanding of this process [45, 46]. Using somewhat different approaches, each group showed that disruption of the C-terminus of β- or γ-ENaC resulted in more channels on the surface of the cell than was the case for the wild-type channels. Firsov et al suggested that these mutations also might affect the gating properties of ENaC [46], but direct evidence for such an effect has been elusive [45]. Excellent progress has been made in our understanding of the mechanism of aberrant surface expression, however.

The discovery of missense mutations involving three amino acids in the distal portion of the C-terminus of β-ENaC has focused attention on this region [47–49]. A stretch of amino acids, PPPXY, is conserved in all three of the ENaC subunits. This domain is similar to that of the LDL receptor (NPXY), which is responsible for internalization. Good evidence now indicates that this region is extremely important in regulating the amount of ENaC complex residing on the cell surface. Mutation of the tyrosine residue (Y) in either the α-, β-, or γ-ENaC subunit increases the sodium current because of increased surface expression [45]. Interestingly, this surface expression phenomenon does not necessarily require the remainder of the ENaC molecule. A chimera consisting of the C-terminus of β-ENaC with another molecule expressed on the cell surface can be equally affected by mutating this tyrosine residue. These experiments point to an extremely important role for this region of ENaC in regulating its activity by surface expression.

Staub and coworkers, using the yeast two-hybrid system, have identified a protein that binds to the PPXY domain of β- and γ-ENaC [50]. This protein, called Nedd4, is expressed in many cell types. Most important, it is expressed in collecting duct cells, pulmonary alveolar cells, and distal colon epithelial cells [51]. Nedd4 contains three WW domains, regions that contain two highly conserved tryptophan residues (W) and consist of 35 to 40 amino acids each [52]. Nedd4 also contains a ubiquitin ligase domain in its carboxy-terminal region. This domain is capable of ubiquitinating a variety of proteins, a process involved in protein degradation. Finally, Nedd4 has a C2 domain, a region involved in calcium-phospholipid binding believed to be involved in endocytosis via a clathrin-AP-2 complex [53]. Other groups have provided strong evidence that Nedd4 is involved in the surface expression of the ENaC complex [54–56].

A schematic interpretation of the interaction of Nedd4 with a model ENaC protein is shown in Figure 6. Binding of the WW domain of Nedd4 to the PPXY region of ENaC produces ubiquitination of a region in the amino terminus of ENaC. Endocytosis might be effected via the C2 domain, which is coupled to a clathrin-mediated process. The molecular details are unknown, but endocytosis of clathrin-coated vesicles depends on the protein dynamin. Shimkets and colleagues showed that injecting a dominant-negative dynamin mutant into oocytes expressing ENaC significantly increased the sodium channel activity and sodium transport [57]. Taken together, these results begin to build a new chapter in the regulation of sodium transport by the collecting duct. The dis-

covery of the genetic defect causing Liddle's syndrome thus is providing important information regarding its pathogenesis and, in addition, insights into the normal regulation of ENaC surface expression.

Additional ways of regulating ENaC activity

Given the importance of ENaC for the viability of the entire organism, it comes as no surprise that its activity is highly regulated. The best-known regulator of its activity is, of course, aldosterone. Despite the fact that we have known for more than 40 years that aldosterone increases the activity of the epithelial sodium channel, we still have very little specific information about the molecular events leading to this increase. One potentially important process is the regulation of transcription of one or more of the subunits [58]. The molecular identification of the channel complex has been a critical step in our understanding of how aldosterone functions. As a result of this discovery, investigators are making excellent progress in understanding how other factors regulate ENaC activity.

Knowing the identity of ENaC has permitted the examination of how this channel is regulated. Our understanding of these factors is just beginning, but some are now known to be involved in direct effects, as opposed to more indirect effects, on the ENaC complex. We shall briefly consider those relating to the role of phosphorylation, intracellular sodium, methylation, and extracellular proteases.

It has long been recognized that the rate of sodium transport across high resistance epithelia such as the CCD could be altered dramatically by a number of vasoactive substances [42]. A common mediator of many of these agents could be kinases, such as protein kinases C and A. However, these kinases do not produce consistent effects on the magnitude of sodium transport; their effects seem to be tissue-specific [59, 60]. So there has been considerable speculation regarding whether the ENaC subunits could be phosphorylated directly. Evidence now suggests that at least two of the ENaC subunits (β- and γ-) can be phosphorylated in vivo [61]. The consequences of this phosphorylation are not yet clear, but some interesting possibilities emerge. First, serines and threonines residing in the carboxy termini were phosphorylated. This observation raises the intriguing possibility that these regions might have multiple sites involved in ENaC regulation. In addition to the PPXY regions, other domains might be involved. In this regard, it is interesting to note that phosphorylated serines or threonines on target proteins might enhance the binding of proteins containing the WW domain, such as Nedd4 [62].

The concentration of intracellular sodium is an important regulatory factor in transepithelial sodium transport. The physiologic role of this feedback mechanism is maintenance of intracellular homeostasis. If the apical membrane ENaC were to increase the entry of sodium without any safeguards (counterregulatory system), the cell would be at risk for a greatly increased intracellular sodium concentration and, consequently, significantly increased cell volume. Despite wide recognition of the importance of this phenomenon, we have gained little understanding of the molecular events leading to this counterregulation. Recently published experiments have begun to examine this question from a new perspective. First, there is good evidence that a G_0 protein is required for ENaC downregulation [63]. The G proteins also have been implicated in the upregulation of ENaC by aldosterone [64]. Second, the counterregulation of ENaC activity by intracellular sodium is defective in ENaC subunits with the Liddle's mutation [65]. Third, the reduction in sodium transport caused by high intracellular $[Na^+]$ might be mediated in part by Nedd4 [56]. The fact that the Liddle's mutations eliminate the major site of Nedd4 binding on ENaC makes this interaction of potential pathophysiologic importance. Finally, although protein kinase C has been implicated in this feedback process [66], its precise role is still unclear.

Carboxymethylation of ENaC was recently demonstrated in vitro [67]. The potential importance of this observation is that one mechanism for the action of aldosterone has long been thought to be via methylation of proteins [68, 69]. The fact that one of the methylated proteins might be ENaC itself increases the number of potential interactions that might lead to regulation of this complex.

We also should consider the newest mechanism whereby the ENaC molecules might be regulated directly. Vallet et al have reported that a serine protease, CAP1, when coexpressed in oocytes with ENaC, increases sodium transport [70]. The protease activity seems to be important, as an inhibitor, aprotinin, can inhibit sodium transport in this system, and trypsin can activate sodium transport. A similar effect of aprotinin has been shown in M1 cells [71], a cell line that is derived from the mouse collecting duct. Because CAP1 is a GPI-anchored protein, it should be available to interact with other apical membrane proteins, perhaps to alter their function in this way. The large and complex extracellular domain of ENaC and related proteins makes such an interaction an attractive one to contemplate.

Do other abnormalities in ENaC cause less severe, salt-sensitive hypertension?

One of the major reasons why we attempt to understand the pathophysiology of rare genetic diseases is to discover how similar, less flagrant but more common disorders might be caused and treated. Hence, many investigators in the field of hypertension are intrigued by the possibility that some people with hypertension have genetic differences in ENaC subunits that produce

more subtle changes in the function of the channel complex, not produce overt hypokalemia, but still contribute to salt-sensitive hypertension. A good rationale exists for us to suspect that an intrinsic defect in ENaC produces increased sodium absorption without hypokalemia. Even some members of a family with the abnormal Liddle's ENaC gene express a mild phenotype [72]. Thus, it is reasonable to presume that mutations producing a milder functional disorder might produce hypertension in the setting of an abundant sodium intake without producing hypokalemia and metabolic alkalosis. Such mild phenotypes even might have provided a selective survival advantage under circumstances in which dietary sodium was meager.

Given this possibility, investigators have begun to examine populations of salt-sensitive hypertensive patients to determine whether indeed such mutations exist in any of the ENaC subunits. Not surprisingly, the search has been concentrated in the carboxy termini of β- and γ-ENaC, because the defects that produce Liddle's syndrome reside in those regions (Fig. 5). To date, several reports have documented polymorphisms in β- and γ-ENaC [73, 74]. However, these polymorphisms do not segregate with hypertension in family members, even though many of them were found more commonly in individuals of African origin. When these variants have been expressed in oocytes, a significant functional difference has not been noted [74].

The search has uncovered one polymorphism that might play a role in salt-sensitive hypertension. Su et al reported that a single amino acid substitution in the β subunit (βT594M) was found in 6% of African-American subjects, but this substitution was not found in their Caucasoid population [75]. Furthermore, this mutation was believed to render amiloride-sensitive sodium currents in lymphocytes unresponsive to phorbol esters and more sensitive to the stimulatory effects of cAMP analogues [75, 76]. The interpretation of these data has been difficult. One question relates to the significance of amiloride-sensitive currents in lymphocytes, as the characteristics of these currents are quite different from those reported in cells expressing ENaC. Lymphocytes might have a different assembly of ENaC or a different sodium channel [77]. The possible contribution of this mutation to hypertension has been evaluated in hypertensive African subjects residing in London. The βT594M mutation occurred more frequently than in normotensive African subjects [78]. Whether this mutation actually plays a role in the pathogenesis of salt-sensitive hypertension in humans will require further investigation.

Salt-sensitive hypertension in rats

The genetic diversity of humans makes unraveling the array of genes that predispose to hypertension a formidable undertaking when there is no readily identifiable phenotype (such as hypokalemia). Genetic diversity can be controlled or eliminated in rodents bred for specific traits, such as hypertension. Many years ago Rapp refined the Dahl salt-sensitive (S) and salt-resistant (R) strains of rat and inbred them [79]. These animals now represent a valuable resource for investigating specific genes that contribute to hypertension. Other inbred strains such as the spontaneously hypertensive rat are similarly valuable, as different genes can interact in various ways to produce hypertension.

Do Dahl S rats have an abnormality in collecting duct sodium transport? To address this question, we have refined the method of measuring sodium transport in primary cultures of inner medullary collecting duct cells. These cells, when grown as monolayers on filters, have sodium transport that is stimulated by steroids and inhibited by amiloride [80]. The pathway for sodium entry in these cells is ENaC [81]. When we measured sodium transport by cultured inner medullary collecting duct cells from Dahl S and R rats, we found that S rat monolayers transported about twice as much sodium as did R rat monolayers [82]. The reason for this difference is that S monolayers permit more sodium entry than do R monolayers [83]. These results point to the possibility that the ENaC genes or factors responsible for their regulation differ in these two strains. To date, no convincing data have implicated genetic abnormalities in ENaC subunits in any rat model of hypertension [84, 85]. However, given the progress in unraveling the factors involved in regulating ENaC, progress in understanding salt-sensitive hypertension in the rat is likely as well.

In summary, the discovery of the ENaC proteins has opened the door to discovering major secrets to the function of the collecting duct and the regulation of sodium balance. It is easy to predict that this family of proteins will continue to amaze us as we discover more family members and explore their biologic diversity. It is safe to assume that we will continue to learn the molecular details of how this complex functions and how the molecules involved in its regulation contribute to disease.

QUESTIONS AND ANSWERS

Dr. Nicolaos E. Madias (*Chief, Division of Nephrology, New England Medical Center, Boston, Massachusetts*): Thank you for a fine presentation. I find it fascinating that by far the largest portion of the ENaC protein is extracellular, being bathed by urine and fecal contents in the case of kidney and colon, respectively. It is tempting to consider that it is there for a cause. Are there any data to support the notion that this segment might harbor a sodium sensor or a regulatory domain that interacts with its environment?

Dr. Stokes: Unfortunately, there is no evidence that the extracellular domain of ENaC acts as a sodium recep-

tor. Nonetheless, endocrinologists who have looked at this channel say that it appears just like a receptor. Many people have been struck, as you have been, by the fact that there is an enormous amount of complexity in the extracellular domain. It is attractive from the physiologic perspective to postulate that something in the urine might be able to regulate the activity of that sodium channel, perhaps something produced by an upstream segment of the nephron. We've looked hard and not been able to uncover anything that we think behaves in that fashion. I would speculate that we will ultimately discover how this large extracellular domain interacts with its environment. An important recent discovery is that the serine protease CAP1 might alter the extracellular domain and thus alter the gating of the channel [70]. I expect that this interaction with the extracellular domain will be the subject of considerable attention over the next few years.

Other members of this family, which may be expressed as single proteins, are not necessarily devoted only to sodium transport as ENaC is. If they are mechanosensors, the extracellular domain can respond to perturbations in the physical environment. Thus, the extracellular domain can transmit a signal causing opening of the channel and allowing sodium, potassium, or calcium into the cell. In that sense, the protein could act as a receptor for an environmental stimulus. I would not be surprised if ENaC functioned similarly.

Dr. John T. Harrington (*Dean, Tufts University School of Medicine, Boston*): I'm also struck by the size of this channel. Does the extracellular loop actively direct sodium movement down to the membrane, or does the loop simply trap sodium in a passive way, the sodium, if you will, then floating down to the membrane?

Dr. Stokes: There's not enough information to answer that question precisely. The cartoons that people like to draw would have that large extracellular domain act like a funnel into which sodium can be directed. At the base of that funnel, the area proximal to the second membrane-spanning domain might act as a specific site for sodium binding and also amiloride binding. It is that site that probably directs the sodium into the channel. The binding of sodium does not require ATP. Everything we know about the ENaC suggests that the magnitude of sodium transport is driven by the electrochemical gradient. It does not need any extra energy to effect sodium transfer from one side to the other. This is a passive process, but it is regulated by the binding sites that line the pore.

Dr. Madias: Can you summarize for us what is known about the mechanism of regulation by aldosterone of the activities of the apical sodium and potassium channels and the basolateral sodium pump? Is there a well-defined temporal sequence in these regulatory functions?

Dr. Stokes: That's a complicated question and I'll answer it in two parts. The first part is that aldosterone in some, but not in all, tissues increases sodium transport in a biphasic fashion. The biphasic response is characterized as an early response and a late response. The early response can be seen within an hour or two. Six to 24 hours later, there is a greater increase in sodium transport. The classic way of thinking about this is that two types of transcriptional responses are occurring, although some evidence suggests that aldosterone produces non-transcriptional responses too [92].

As your question implies, good evidence suggests that aldosterone increases the magnitude of sodium transport by increasing the entry of sodium through ENaC and by increasing the activity of the Na^+/K^+ ATPase. Aldosterone's effect on the latter is modest and poorly understood. Its effect on ENaC is still under intense investigation, but important clues have recently emerged. One of the clearest and best-described actions of aldosterone is that it increases the transcription of one or more of the ENaC subunits. In the kidney and lung, steroids increase the mRNA levels of α-EnaC, while in the colon, steroids increase the levels of β- and γ-EnaC mRNA [37]. It is likely that this increase largely reflects an increase in the rate of transcription [58]. However, why the response in the colon differs from the response in the kidney and in the lung is unknown.

Another mystery involves the fact that in the kidney the increase in the mRNA level of α-ENaC does not necessarily correspond with the increase in sodium absorption. Rats fed a low-NaCl diet increase the rate of sodium transport in the cortical collecting duct, but ENaC mRNA levels are unchanged [37]. In the inner medulla, the correlation between the magnitude of sodium transport and α-EnaC mRNA is much tighter [37, 80, 81].

There has been quite a bit of effort directed at trying to discover other aldosterone-induced proteins. Recently three proteins have been described that are induced by aldosterone and are reasonable candidates for regulation of the ENaC and/or Na^+/K^+ ATPase activity.

The first protein, CHIF (channel-inducing factor), was cloned from the rat colon and is regulated by potassium balance and aldosterone [93]. Unfortunately, we don't know how that protein might interact with ENaC. The second protein is a RAS family member: Kras 2. It is induced by aldosterone in A6 cells, an amphibian cell line [94]. Its role in the regulation of ENaC is not clear. The third protein, sgk (serum and glucocorticoid-regulated kinase) [95], was discovered a few months ago to be an aldosterone-induced protein. It was originally cloned from mammary epithelial cells exposed to serum and glucocorticoids. It is called a kinase because it looks like a kinase. To date, there is no direct proof that it actually is a kinase, and its substrate is unknown. Nevertheless, this protein is induced by steroids in epithelial

cells, and when co-injected into *Xenopus* oocytes with ENaC, sodium transport is increased. Sgk is a promising candidate molecule for mediating the action of aldosterone.

Dr. Harrington: Is there exact structural similarity between the renal epithelial sodium channel and the sodium channel in other cells? Second, in patients with Liddle's syndrome, are the functional defects within renal epithelial cells expressed in non-renal tissues?

Dr. Stokes: The primary sequence of α-, β-, and γ-ENaC subunits in kidney, colon, and lung is identical. We are not yet sure about the stoichiometry of the channel. One view is that the stoichiometry is 2α:1β:1γ [13, 14]. Another view is that the stoichiometry is 3α:3β:3γ [15, 16]. One can experimentally manipulate the oocyte expression system so that more of one kind of subunit is expressed than the others. Depending on this relationship, one can get different characteristics of the sodium channel [17]. Nobody will be surprised if the function of the sodium channel in lung, sweat gland, salivary gland, or colon might be slightly different than in the collecting duct of the kidney, depending in part on how these subunits are assembled. We have recently discovered a striking example of heterogeneity of ENaC subunits in uroepithelial cells. There are large amounts of β- and γ-ENaC mRNA from the renal pelvis to the urinary bladder, but there is almost no trace of α-ENaC [20]. The urinary bladder epithelium is not known to absorb a lot of sodium. We are not sure how these subunits function, or whether they form a channel. We think that they might play a role in mechanosensation and might participate in sensing the amount of distention in the ureters, pelvis, and bladder.

Dr. Madias: I have a question on each of the three types of pseudohypoaldosteronism. Regarding the autosomal-recessive type-I PHA, are there any functional consequences of the heterozygous state under conditions of stress, such as low-sodium diet?

Dr. Stokes: I'm not aware of any published studies in this regard, but the hypothesis that people are studying is that these patients, when they are put under stress, demonstrate a modest but measurable defect in their ability to retain sodium or excrete potassium. They also can have elevated baseline levels of aldosterone and renin, so they might be chronically volume contracted.

Dr. Madias: Concerning the second type, I agree that the designation of PHA is a misnomer. Do patients with this syndrome respond to mineralocorticoids? Is there any recent evidence to support or refute the "chloride shunt" hypothesis as the cause of the syndrome?

Dr. Stokes: The answer is complicated by the fact that type-II PHA is found in a heterogeneous group of patients. The best-studied group was presented by Gordon [2]. These patients do respond to mineralocorticoid hormones.

With regard to the question of the "chloride shunt" hypothesis, we will have to wait for the identification of the affected genes. Progress has been made in this area, as regions of chromosomes 1 and 17 have been implicated in a group of families with this syndrome [96]. At present, discussions regarding an enlarged "chloride shunt" must be considered speculative.

Dr. Madias: Regarding the third type of PHA, would you reflect on the potential basis of aldosterone resistance in these patients?

Dr. Stokes: Type-III PHA is more common in clinical medicine than is type-I or type-II. The syndrome is a consequence of one or more factors that render the collecting duct insensitive to aldosterone. One possibility that is attractive to us based on some work in our laboratory is that disorders such as obstruction, medullary cystic disease, or inflammation produce increasing amounts of TGF-β. We know from our previous studies that TGF-β renders the collecting duct insensitive to aldosterone [97]. I would not be surprised if patients with these disorders have increased production of a factor such as TGF-β that would inactivate the aldosterone-response system.

Dr. Harrington: I'm still having trouble with the type-I PHA story. While the patients have terrible problems in the first years of life, they subsequently do well. I don't understand what changes occur at ages three to six years and what regulates the changes that result in the patient becoming healthy.

Dr. Stokes: I don't understand that either. I think that patients with autosomal-dominant type-I PHA (like our Patient 1) are trying to tell us something. Certainly, it has something to do with the maturation of the human collecting duct, the human mineralocorticoid receptor, and the apparatus responsible for responding to aldosterone.

Dr. Harrington: Why did Grant Liddle's patient go into renal failure 29 years later? Did it have anything to do with Liddle's syndrome, or did she develop another renal disease?

Dr. Stokes: This patient was reported twice, the first time by Liddle and coworkers [40] and the second time by Botero-Valez et al [41]. The latter report suggests that she developed renal failure from hypertension. No other renal disease was evident at the time of transplantation. The success of the blood pressure control following successful transplantation underscores the importance of the kidney in the pathophysiology of hypertension in Liddle's syndrome.

Dr. Madias: Obviously, a number of sodium-transporting proteins could contribute to the pathogenesis of hypertension. Could you summarize for us the available knowledge in experimental models of hypertension? Also, what about abnormalities in these molecules as a contributor to salt resistance?

Dr. Stokes: Inbred rat strains are valuable models for sorting out the molecules responsible for the development of hypertension. Many laboratories are using genetic approaches to discover precisely which genes produce hypertension in which strains of rats. There are a couple of examples of success. The best one has to do with the discovery that 11-β hydroxylase is different in the Dahl salt-sensitive and -resistant strains [98]. This enzyme produces more 18-hydroxy-DOC, a weak mineralocorticoid, in the Dahl salt-sensitive rat than in the Dahl salt-resistant rat. When this was discovered, an enormous amount of activity was directed at looking for low-renin, low-aldosterone patients in whom a cryptogenic steroid might be acting as a mineralocorticoid. It has been difficult to convince anyone that this is a major cause of low-renin hypertension in humans. Nevertheless, this is one example in which a single protein clearly has been shown to be responsible, at least in part, for salt-sensitive hypertension in the rat.

Other candidate genes exist for hypertension in the rat. One of the most persuasive candidates is the protein adducin, which has sequence differences between the salt-sensitive and salt-resistant Milan rat strains [99]. Bianchi and coworkers proposed that this cytoskeletal protein is altered in the salt-sensitive strain such that renal tubular sodium absorption is inappropriately high.

The discovery of genes predisposing to salt resistance is difficult from a methodologic perspective. Some investigators have proposed that salt sensitivity, like blood pressure itself, represents a continuous spectrum. In some subjects, the magnitude of the increase in blood pressure on a high-salt diet is small, and sometimes it is large. This phenomenon is true for rats as well as humans. It seems to me that the best approach to discovering genes promoting salt resistance is to discover differences between genetically similar salt-sensitive and salt-resistant rats, and study the genes systematically.

Dr. Madias: You mentioned that there could be redundant proteins that might explain why some humans have survived disruption of one of the ENaC subunits and mice cannot. Are there any candidates?

Dr. Stokes: Yes, there is a candidate. Waldmann and coworkers have cloned a family member from human kidney that is also found in brain, pancreas, testis, and ovary [100]. They called this family member δ-ENaC because it had the strongest homology to the ENaC part of the family (Fig. 3). When expressed in oocytes alone, it produced a small sodium current, much as α-ENaC does. There are differences between δ-ENaC and α-EnaC, but δ-ENaC is able to substitute for α-ENaC when coexpressed with β- and γ-ENaC. The single channel conductance, amiloride sensitivity, and Li^+/Na^+ selectivity for δ-ENaC are different than for α-ENaC. Thus, this gene product could rescue some human conditions in which α-ENaC is defective. The physiologic significance of δ-ENaC is unknown.

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